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SPECIFICATION FOR BLACK PEPPER ELEORESIN

(*Second Revision*)

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Indian Standard

SPECIFICATION FOR BLACK PEPPER OLEORESIN

(Second Revision)

0. FOREWORD

0.1 This Indian Standard (Second Revision) was adopted by the Indian Standards Institution on 30 April 1984, after the draft finalized by the Spices and Condiments Sectional Committee had been approved by the Agricultural and Food Products Division Council.

0.2 Spices in the form of oleoresins are increasingly used by food, pharmaceutical and flavour industries. The oleoresins are preferred as their quality, flavour and strength can be standardized, for better uniformity of the end product.

0.3 Black pepper oleoresin is obtained by extraction of black pepper with food grade solvent and subsequent careful removal of the solvent by distillation. It is a mixture of essential oil (oil of pepper), the pungent principle and other resinous materials. The presence of chlorophyll gives it a dark green colour, but decolourization may be done to get a light-coloured oleoresin. It is extensively used for flavouring foodstuffs.

0.4 This standard was first issued in 1970 and was first revised in 1975 to include the latest provisions for solvents used and requirements for essential oil content refractive index, etc. Based on further data, this second revision includes the solvent of ethyl acetate and solvent grade hexane.

0.5 This standard is subject to the restrictions imposed under the *Prevention of Food Adulteration Act*, 1954, and the Rules framed thereunder, wherever applicable.

0.6 For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS : 2-1960*. The number of significant places retained in the rounded off value should be the same as that of the specified values in this standard.

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*Rules for rounding off numerical values (*revised*).

1. SCOPE

1.1 This standard prescribes the requirements and the method of sampling and test for black pepper oleoresin.

2. TERMINOLOGY

2.1 For the purpose of this standard, definitions given under **2** of IS : 326-1968* shall apply.

3. REQUIREMENTS

3.1 Raw Material — Black pepper oleoresin shall be obtained from the ground dried berries of the vine *Piper nigrum* L.

3.2 Solvents — The following solvents, either singly or in mixture, shall be used. As far as possible, they should conform to the specifications indicated against each:

a) Isopropanol (*See* IS : 2631-1976†)
b) Trichloroethylene (*see* IS : 245-1970‡)
c) Acetone (*see* IS . 170-1976§)
d) Ethylene dichloride (*see* IS : 869-1976||)
e) Rectified spirit (*see* IS : 323-1959¶)
f) Ethyl acetate (*see* IS : 229-1972**)
g) Solvent grade hexane.

3.3 Description — The material shall be either a viscous dark-green to light brown dispersion or shall consist of an upper oily layer and a lower crystalline mass, free from extraneous matter and adulterants.

3.4 The material shall also comply with the requirements given in Table 1.

4. PACKING AND MARKING

4.1 Packing — The material shall be supplied in tightly closed glass, pure aluminium or suitable lined containers or containers of high density polyethylene food grade, which shall be nearly full.

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*Methods of sampling and test for natural and synthetic perfumery materials (*first revision*).

†Specification for isopropyl alcohol (*first revision*).

‡Specification for trichloroethylene, technical (*second revision*).

§Specification for acetone (*second revision*).

||Specification for ethylene dichloride (*second revision*).

¶Specification for rectified spirit (*revised*).

**Specification for ethyl acetate (*second revision*).

TABLE 1 REQUIREMENTS FOR BLACK PEPPER OLEORESIN

(*Clauses* 3.4 *and* 6.1)

Sl No.	Characteristic	Requirement	Method of Test, Ref to	
			Appendix	Cl No. in IS : 326-1968*
(1)	(2)	(3)	(4)	(5)
i)	Odour and taste	Typical of black pepper; no off-odours or off-flavours due to residual solvent or other causes	A	—
ii)	Essential oil			
	a) Content percent (*v/m*)	18 to 35	—	16·7
	b) Specific gravity at 30°C/30°C	0·852 to 0·879	—	5
	c) Optical rotation at 30°C	– 1° to – 28°	—	6
	d) Refractive index at 20°C	1·473 to 1·483 (*see* Note)	—	7
iii)	Piperine content, percent by mass, *Min*			
	a) By Kjeldahl's method	55	B-1	—
	b) By spectrophotometric method	40	B-2	—
iv)	Residual solvent, mg/kg, *Max*			
	a) Acetone, ethylene dichloride or trichloroethylene	30	C	—
	b) Isopropanol, ethyl acetate	50		
	c) Hexane	25		

NOTE — Correction factor for refractive index is 0·000 4.

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*Methods of sampling and test for natural and synthetic perfumery materials (*first revision*).

4.1.1 The material shall be protected from light and stored in a cool place.

4.2 Marking — The containers shall be marked with the following particulars:

a) Name of the material;

b) Manufacturer's name and trade-mark, if any;
c) Net mass of the material when packed; and
d) Batch or code number.

5. SAMPLING

5.1 Representative samples of the material, sufficient to give a composite sample for triplicate determinations, shall be drawn from the containers selected from the lot as prescribed under **3** of IS : 326-1968*.

5.2 Tests for all the characteristics shall be conducted on the composite sample.

5.3 Criterion for Conformity — The lot shall be declared to have conformed to this specification if the results of tests on composite sample meet the corresponding requirements.

6. TEST METHODS

6.1 Tests shall be conducted as prescribed in col 4 and 5 of Table 1.

6.2 Quality of Reagents — Unless specified otherwise, pure chemicals and distilled water (*see* IS : 1070-1977†) shall be employed in tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the results of analysis.

APPENDIX A

[*Table* 1, *Item* (i)]

ASSESSMENT OF ODOUR AND TASTE

A-0. GENERAL

A-0.1 Outline of the Method — The odour and taste of the material are evaluated by comparing with a prepared standard oleoresin.

A-1. PROCEDURE

A-1.1 Preparation of Standard Oleoresin of Black Pepper — Separate the oil of pepper from ground black pepper by steam distillation. Extract the dry residue so obtained with 95 percent ethanol and distil off ethanol completely, using high vacuum towards the end. Mix the

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*Method of sampling and test for natural and synthetic perfumery materials (*first revision*).

†Specification for water for general laboratory use (*second revision*).

oil of pepper already obtained with the residue and homogenize the mixture. This two-step procedure avoids loss of essential oil during solvent removal and high vacuum helps to remove last traces of alcohol.

A-1.2 Odour — Test the odour quality of the sample by comparing with the standard oleoresin by smelling strip technique as prescribed under **4** of IS : 2284-1963*.

A-1.2.1 The material shall be deemed to have passed the test if it gives no off-odours but only the typical odour of black pepper.

A-1.3 Taste — Weigh out in a beaker 4·5 g of the neutral soup base containing 18 parts of sodium chloride, 22 parts of castor sugar and 60 parts of corn flour. Mix into a smooth cream with 10 ml of cold water. Add, while stirring well, 100 ml of boiling water. Place the beaker on a hot-plate and bring the soup to boil for one minute. Add one gram of sample and stir thoroughly. Cool to about 60°C and submit to tasting panel for evaluation. A similar soup mixed with the standard oleoresin shall be prepared for composition.

A-1.3.1 The material shall be deemed to have passed the test if it has no off-flavours.

APPENDIX B

[*Table* 1, *Item* (iii)]

DETERMINATION OF PIPERINE CONTENT

B-0. GENERAL

B-0.1 Outline of the Method — Two methods for estimation of the piperine content have been prescribed. It is estimated either by the Kjeldahl's method or spectrophotometrically. The Kjeldahl's method estimates nitrogen and express this as piperine and so is subject to variations due to the presence of other nitrogen containing compounds. This method, therefore, does not detect the presence of extraneous nitrogenous substances. The spectrophotometric method is more reliable, and the specificity of this lies in the fact that other nitrogenous substances present in the oleoresin do not interfere in the determination.

NOTE — Either of the two methods may be used but in case of dispute the spectrophotometric method shall be used as the referee method.

---

*Method for olfactory assessment of natural and synthetic perfumery materials.

B-1. KJELDAHL'S TOTAL NITROGEN METHOD

B-1.1 Apparatus

B-1.1.1 *Kjeldahl Digestion and Distillation Apparatus*

B-1.1.2 *Kjeldahl Flask* — 800 ml capacity.

B-1.1.3 *Receiving Flask* — 500 ml capacity.

B-1.2 Reagents

B-1.2.1 *Mercury*

B-1.2.2 *Concentrated Sulphuric Acid* — sp gr 1·84 (*see* IS : 266-1961*).

B-1.2.3 *Sodium Sulphate* — *see* IS : 255-1977†.

B-1.2.4 *Sodium Sulphide Solution* — 4 percent (*w/v*).

B-1.2.5 *Sodium Hydroxide Solution* — 45 percent (*w/v*).

B-1.2.6 *Standard Sodium Hydroxide Solution* — 0·1 N.

B-1.2.7 *Standard Hydrochloric Acid* — 0·1 N.

B-1.2.8 *Methyl Red Indicator Solution* — Prepared by dissolving 0·1 g in 100 ml of rectified spirit (*see* IS : 323-1959‡).

B-1.3 Procedure — Weigh accurately 1 g of the material and place it in a 800 ml Kjeldahl flask. Add 0·65 g of mercury, 17 g of sodium sulphate, 25 ml of 93 to 98 percent sulphuric acid and a few boiling chips. Place the flask in inclined position and heat gently until frothing ceases, if necessary add small amount of paraffin to reduce frothing, boil briskly until solution is clear and then continue boiling for additional 1 hour. Cool, add about 250 ml of water and shake well to dissolve solid sulphates. Add 50 ml of sodium sulphide solution to precipitate mercury. Add 80 ml of aqueous sodium hydroxide solution and connect quickly to the condenser without agitation. Distil at least 150 ml into a 500 ml Erlenmeyer flask containing 50 ml of 0·1 N hydrochloric acid and about 100 ml of water (make sure that the end of the condenser is immersed in the standard acid solution in the receiver). Titrate excess acid with 0·1 N sodium hydroxide solution using methyl red indicator to yellow end point. Carry out a blank test.

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*Specification for sulphuric acid (*revised*).

†Specification for sodium sulphate, anhydrous (*first revision*).

‡Specification for rectified spirit (*revised*).

B-1.4 Calculation

$$\text{Piperine content, percent by weight} = 2{\cdot}851\,\frac{(V_2 - V_1)}{W}$$

where

V_2 = volume in millilitres of 0·1 N sodium hydroxide solution required in the blank test,

V_1 = volume in millilitres of 0·1 N sodium hydroxide solution required in the test, and

W = weight in grams of the material taken for the test.

B-2. SPECTROPHOTOMETRIC METHOD

B-2.1 Apparatus

B-2.1.1 *Spectrophotometer* — any suitable model, with attachment for ultra-violet radiation and 1-cm silica cells.

B-2.2 Reagents

B-2.2.1 *Benzene* — reagent grade (*see* IS : 1840-1961*).

B-2.2.2 *Piperine* — pure. It may be prepared as follows:

> Add 500 ml of petroleum ether to 100 g of black pepper oleoresin. Stir well and filter. Dissolve the residue in a minimum of hot absolute ethanol, filter and allow the crude piperine to crystallize out as long needles. Recrystallize the piperine at least three times from hot absolute ethanol. Dry the crystals in vacuum at 60°C for 6 hours.

NOTE — Melting point of pure piperine is 128°C to 129°C.

B-2.3 Procedure — Weigh accurately 0·2 to 0·3 g of the material, transfer to a 100 ml volumetric flask and make up to mark with benzene. Pipette out 0·5 ml aliquot into another 100 ml volumetric flask and make up to mark with benzene. Immediately transfer a portion of this solution to a 1 cm cell, using a matched cell containing redistilled benzene as the reference. Observe the absorbance value at 345 mμ in the spectrophotometer. Refer to the calibration curve (*see* Note) for piperine in benzene to obtain the percent concentration in the final solution and make the necessary correction for the aliquot taken to obtain the percentage of piperine in the original sample.

NOTE — The calibration curve shall be obtained as follows:

Prepare solutions of pure piperine in different concentrations ranging from 250 to 1 250 μg in 100 ml of benzene. Read the absorbance value of each concentration in the spectrophotometer at 345 mμ. Plot the graph of concentration against observed absorbance.

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*Specification for benzene, reagent grade.

APPENDIX C

[*Table* 1, *Item* (iv)]

DETERMINATION OF RESIDUAL SOLVENTS

C-0. GENERAL

C-0.1 Outline of the Method — The sample is chromatographed where the residual solvents are separated and shown as peaks in the chart recorder. From the height and width of the peaks, amount of solvent signified by each peak may be calculated.

C-1. APPARATUS

C-1.1 A gas chromatographic unit satisfying the following requirements:

a) It shall be provided with differential type detection device and recorder.

b) It shall be provided with separate thermostats for the columns and the cell. The columns shall be maintained at a constant temperature between 70°C and 80°C. The temperature of the cell shall be kept constant preferably at 150°C.

c) Each column shall be 2 to 2·5 m in length and shall contain about 50 to 80 ml of the liquid phase.

d) Both the columns shall have impregnated packing consisting of 20 percent by mass of a liquid phase such as Ucon LB — 135 on 35 — to 80 — mesh fire brick. Injection of sample is done in one column while the other acts as the reference in a dual column arrangement.

e) Carrier gas in the unit shall be hydrogen.

f) The flow rate of the carrier gas shall be 50 to 70 ml per minute.

C-1.2 A volatile oil determination apparatus with a trap for oils heavier than water (*see* **16.7** of IS : 326-1968*).

C-1.3 Pipettes, Volumetric Flasks and Heaters.

C-2. REAGENTS

C-2.1 Toluene — Purity of the toluene for the purpose of this analysis may be determined by gas chromatographic analysis using the conditions prescribed in **C-2.6.1** and injecting the same quantity of toluene as will be injected in the analysis for solvents. If impurities interfering with this test are present in the toluene, they will appear as peaks prior to the toluene and may be removed by fractional distillation.

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*Methods of sampling and test for natural and synthetic perfumery materials (*first revision*).

C-2.2 Potassium Carbonate, Anhydrous

C-2.3 Sodium Sulphate, Anhydrous

C-2.4 Detergent and Antifoam — both free of volatile compounds. If volatile compounds are present, they may be removed by lengthy boiling of their aqueous solutions. Other anti-bumping compound may also be used.

C-2.5 Benzene — analytical reagent grade. Purity shall be determined as in the case of toluene.

C-2.6 Reference Solutions

C-2.6.1 *Benzene Solution* — Prepare a solution containing 2·85 ml of benzene per litre of toluene. If the toluene available contains benzene as the only impurity, the benzene level in the toluene may be determined and sufficient benzene added to bring the level to that stated above. A procedure for determining the benzene content of toluene is as follows:

> At constant carrier gas flow rate and column temperature, with other instrumental conditions constant, determine the area of benzene in a suitable sample size of toluene. Add known amounts of benzene to this toluene and determine the areas for each known amount under the same conditions. Plot the results on linear graph paper, with the known concentration of benzene as the ordinate and area as the abscissa. The plot should be linear, and the negative intercept of the ordinate will give the concentration of benzene in the original toluene.

C-3. CALIBRATION OF INSTRUMENT

C-3.1 Determine the response of the detector in the instrument for known quantities of solvent, by injecting known mixtures of solvents and benzene in toluene. The levels of the solvents and benzene in toluene should be of the same magnitude as they will be present in the analysis.

C-3.2 Calculate the areas of the solvents in relation to benzene. Compute the calibration factor of the detector for any particular solvent as follows:

$$C = \frac{\text{mass percent of the solvent}}{\text{mass percent of benzene}} \times \frac{\text{area of benzene}}{\text{area of the solvent}}$$

C-3.2.1 The recovery of the various solvents from the oleoresin, in relation to the recovery of benzene, by the distillation procedure is as follows:

	Percent (m/m)
Hexane	52
Acetone	85
Isopropanol	100
Methylene chloride	87·5
Trichloroethylene	113
Ethylene dichloride	102
Ethyl acetate	42

C-4. PROCEDURE

C-4.1 Preparation of Sample Solution — Place 50·0 g of the oleoresin, 1·00 ml of benzene solution, 10 g of sodium sulphate, 50 ml of water, and a small amount of detergent and antifoam in a 250-ml flask. Attach distilling head and receiver, and collect approximately 15 ml of the distillate. Add 15 g of potassium carbonate to the distillate, cool while shaking and allow the two phases to separate. All the solvents will be present in the toluene layer.

C-4.2 Inject the sample in the column for separation of the different solvents. Elution order on this column is as follows:

> Hexane plus acetone, methylene chloride, isopropanol, benzene, ethyl acetate, ethylene dichloride, trichloro ethylene, toluene.

C-4.3 Inject the sample solution (*see* **C-4.1**) which is sufficient in size to give an accurately measurable deflection of the internal standards of benzene in the toluene phase or of acetone in the aqueous phase. If a katharometer is used as a detector, approximately 40 µl will give adequate deflection. If flame ionization detector is used, approximately 0·4 µl or less will be sufficient. Measure the peak areas of the internal standards and solvents.

C-5. CALCULATION

C-5.1 The level of the internal standards related back to the 50 g of the oleoresin used is:

benzene 43·4 ppm

The level of the residual solvents using benzene as an internal standard is computed as follows:

$$\text{Residual solvent, ppm} = \frac{43{\cdot}4\, C \times 100}{\begin{array}{c}\text{percent recovery}\\ \text{of the solvent}\end{array}} \times \frac{\text{Area of the solvent}}{\text{Area of benzene}}$$

where

C = calibration factor of the defector for the particular solvent (*see* **C-3.2**).

(*Continued from page* 2)

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